12.1.2 Periphyton

Periphyton are algae that live attached to or in close proximity of the stream bottom. Although other plants may occupy the stream benthos, notably mosses and "higher plants" (macrophyton), algae contribute more to the diversity and productivity of Montana streams, particularly streams in the mountainous region of the state.

Periphyton algae may form colonies or filaments that are visible to the unaided eye, or they may be one-celled, microscopic plants that are visible only in their accumulated growth. Two basic types of algae are found in Montana streams: diatoms (Division Chrysophyta, Class Bacillariophyceae) and soft-bodied algae. Soft-bodied algae are represented by four major divisions: green algae (Chlorophyta), blue-green algae or cyanobacteria (Cyanophyta), golden-brown algae (Chrysophyta), and red algae (Rhodophyta).

Pigmented growths of bacteria and fungi (i.e., iron bacteria, "yellow boy", and "sewage fungus") are sometimes found in Montana waters. These growths typically include one or more species of algae interspersed within their matrix. The diverse community of algae, fungi, bacteria and microinvertebrates (nematodes, protozoa, rotifers, etc.) that forms a slime or film coating the stream bottom is called the *Aufwuchs*. Sometimes this community of autotrophs and heterotrophs is also called "periphyton".

For more information about periphyton, the advantages of using benthic algae in stream surveys, and collection and bioassessment methods, refer to APHA et al. (1992), Plafkin et al. (1989), Porter et al. (1993), and Britton and Greeson (1989).

A. INDEX PERIOD

Although stream periphyton may be assessed anytime of the year, the recommended time is summer (June 21 to September 21). This is a time of stable flows and peak periphyton diversity and standing crop in most Montana streams. Summer is also the season most amenable for field work in Montana and the season during which most reference data have been collected.

High flows and turbid waters should be avoided because they limit access to and obscure visibility of the stream bottom. Assessments should be delayed for at least two weeks following high, bottom-scouring streamflows to allow for recolonization by algae and succession to a mature periphyton community. It may be necessary to sample outside the summer period to coincide with flows in ephemeral or dewatered streams, or to track seasonal changes in the biointegrity. When monitoring for trends from year to year, minimize the between-year variance by performing the assessments on or about the same date each year.

B. SITE SELECTION

Selection of sampling locations depends largely on the objectives of the assessment. Factors to consider include access, location of contaminant sources, length of mixing zones, and dilution and attenuation of pollutants.

A reference or control site should be established for each set of study sites. The reference site should be comparable to the study site(s) in terms of depth, gradient, canopy cover (shading), substrate, and other physical features. It should be located in the same reach as the study site or in a local tributary to the study stream that has the same stream order as the reach containing the study site(s). As used here, a "reach" is a section of stream having a uniform stream order. Control sites should be minimally disturbed compared with other sites in the watershed. For long-term monitoring, the control site should be afforded sufficient protection to maintain existing water quality.

The extent of the study site depends on the type of stream to be assessed. For high-gradient streams (see Section 12.1.3 Macroinvertebrate for definition), the sampling site for periphyton is a single riffle. For low-gradient streams, the sampling site is a reach of at least one meander length or about 20 bankful channel widths.

12.1.2.1 Field Observations

The general composition, amount, color and condition of aquatic plants may be assessed in the field using the Aquatic Plant Field Sheet (APFS)(Section 21.1.1.8). This information shall help to describe the health and productivity of the aquatic ecosystem, define nuisance aquatic plant problems, identify potential sources and causes of pollution, and document changes in the plant community over time.

Completing the APFS is equivalent to an RBP Level I assessment for aquatic plants. The APFS should be filled out before completing more detailed assessments of periphyton standing crop, composition and community structure.

12.1.2.2 Field-Based Rapid Periphyton Survey

Semi-quantitative assessments of benthic algal biomass and taxonomic composition can be made rapidly with a viewing bucket marked with a grid and a biomass scoring system. The advantage of using this technique is that it enables rapid assessment of algal biomass over larger spatial scales than substrate sampling and laboratory analysis. Coarse-level taxonomic characterization of communities is also possible with this technique. This technique is a survey of the natural substrate and requires no laboratory processing, but hand picked samples can be returned to the laboratory to quickly verify identification. It is a technique developed by Stevenson and Rier.

PROCEDURE

1. Fill in top of Rapid Periphyton Survey (RPS) Field Sheet.

2. Establish at least three transects across the habitat being sampled (preferably riffles or runs in the reach in which benthic algal accumulation is readily observed and characterized).

3. Select three locations along each transect (e.g., stratified random locations on right, middle, and left bank).

4. Characterize algae in each selected location by immersing the bucket with 45-intersection grid in the water. The original procedure calls for a 50-dot grid, but DEQ modified so our existing percent fines buckets, SOP section 11.8.6, could be used.

   -First, characterize macroalgal biomass.

   -Observe the bottom of the stream through the bottom of the viewing bucket and count the number of intersections that occur over macroalgae (e.g., Cladophora or Spirogyra) under which substrates cannot be seen. Record that number and the kind of macroalgae under the intersections on RPS field sheet.

   -Measure and record the maximum length of the macroalgae.

   -If two or more types of macroalgae are present, count the intersections, measure, and record information for each type of macroalgae separately.

   -Second, characterize microalgal cover.

   -While viewing the same area, record the number of

intersections under which substrata occur that are suitable size for microalgal accumulation (gravel >2 cm in size).

-Determine the kind (usually diatoms and blue-green algae) and estimate the thickness (density) of microalgae under each intersection using the following thickness scale:
0 – substrate rough with no visual evidence of microalgae
0.5 – substrate slimy, but no visual accumulation of microalgae is evident
1 – a thin layer of microalgae is visually evident
2 – accumulation of microalgal layer from 0.5-1mm thick is evident
3 – accumulation of microalgae layer from 1mm to 5mm thick is evident
4 – accumulation of microalgal layer from 5mm to 2cm thick is evident
5 – accumulation of microalgal layer greater than 2cm thick is evident
Mat thickness can be measured with a ruler.

-Record the number of intersections that are over each of the specific thickness ranks separately for diatoms, blue-green algae, or other microalgae.

5. Statistically characterize density of algae on substrate by determining:

-total number of grid points (intersections) evaluated at the site (D_1)
-number of grid points over macroalgae (D_m)
-total number of grid points over suitable substrate for microalgae at the site (d_t)
-number of grid points over microalga of different thickness ranks for each type of microalga (d_i)
-average percent cover of the habitat by each type of macroalgae (i.e., $100X\ D_m/D_t$)
-maximum length of each type of macroalgae
-mean density (i.e., thickness rank) of each type of macroalgae on suitable substrate (i.e., $\Sigma d_i r_i/d_t$); maximum density of each type of macroalgae on suitable substrate

6. QA/QC between observers and calibration between algal biomass (chl *a*, AFDM, cell density and biovolume cm-2 and taxonomic composition) can be developed by collecting samples that have specific microalgal rankings and assaying the periphyton.

12.1.2.3 Standing Crop / Chlorophyll (Sampling, Sample Analysis, Criteria)

The standing crop of periphyton in a stream is controlled by season, nutrient concentrations, current velocity, grazing, shading, water temperature, and other factors. Heavy growths of algae generally indicate inferior water quality.

Excess periphyton growth may clog water filters and irrigation equipment, cause taste and odor problems in water supplies, reduce instream dissolved oxygen levels, interfere with fish spawning, degrade macroinvertebrate habitat, trap sediment and deflect streamflows, and impair the overall aesthetics of a stream.

A. SAMPLING

Periphyton standing crop may be quantified by measuring the amount of accrual on natural substrates at the study site. The use of artificial substrates is not recommended.

Protocol I:

Several techniques are available for sampling periphyton growth from natural substrates (Britton and Greeson (1989), APHA et al. (1992), Porter et al. (1993)). Different techniques may be needed for different substrates, i.e., rocks and sediment.

Periphyton growth tends to be patchy rather than uniform. The heaviest, most problematic accumulations should be targeted for sampling. An additional, more random, sampling procedure is outlined in protocol II. The percent cover by light, moderate and heavy growths can be estimated on the Aquatic Plant Field Sheet. Replicate samples should be collected to determine variability within the study site.

Protocol II:

Direct extraction can also be used to detect chlorophyll *a* (Cattaneo, 1991). Either of the two additional techniques that follow may be used for sampling chlorophyll *a*. First, when taking chlorophyll *a* samples in conjunction with benthic invertebrate sampling, Arocks with algal cover representative of the invertebrate sampling locations are chosen without intentional bias; extreme conditions, such as extremely dense or sparse algal cover are avoided≅ (USGS Open File Report 93-409,14). Sampling a wide variety of habitats is possible. Second, the transect survey technique will allow samples to be taken repeatedly along the same transect line every time a stream is surveyed. (Stevenson, 1997 preliminary draft). This is especially useful for fixed station monitoring in order to detect trends. For the best ability to detect trends, first objectively establish three transects across the habitat being sampled. Collect a six-rock sample along each transect, keeping the samples separate. The samples should be an even representation along the line. For intensive surveys only one transect line is required. To sample, place a minimum of six submerged rocks into a

plastic freezer bag. The rock size should be kept to a minimum so the amount of acetone can be kept to a minimum (flat rocks with an area similar to golf balls and less). Immediately store the sample on ice and away from light (Cattaneo,1991). The samples should be sent to the lab as soon as possible for chlorophyll analysis.

B. ANALYSIS

Protocol I:

Periphyton material collected from a known area of natural substrate should be analyzed for chlorophyll *a* following Standard Methods for Examination of Water and Wastewater (APHA et al. (1992)). Results should be expressed in milligrams of chlorophyll *a* per square meter of substrate.

Protocol II:

Conduct work with chlorophyll in subdued light to avoid degradation. The pigments are extracted from the plankton concentrate from aqueous acetone and the optical density (absorbance) of the extract is determined with a spectrophotometer. The ease with which the chlorophylls are removed from the cells varies considerably with different algae.

Extraction procedure

1) If processing must be delayed, hold samples on ice or at 4^{o}C and protect from the exposure to light. Samples taken from water having a pH 7 or higher may be placed in airtight plastic freezer bags and stored frozen for 3 weeks. Samples from acidic water must be processed promptly to prevent chlorophyll degradation.
2) Place sample in plastic freezer bag and cover with aqueous acetone.* Try to keep the volume of acetone to a minimum (less than 350 mL). Shake the rocks for 30 seconds. Wait one hour and shake again for 30 seconds.

* One variation replaces acetone with methanol (MeOH) (Holm-Hansen, 1978).

3) Let stand for 23 hours, then have the extracts read in a spectrophotometer at wavelengths at 750nm, 665nm, 664nm, 647nm, and 630nm.

Measuring surface area of stones

1) Measure 25cm^2 of aluminum foil and weigh to have a weight-to-area ratio.
2) Wrap the rocks with aluminum foil and trim off the excess foil from the rock.
3) Weigh the foil and use the ratio to find the surface area of the rocks.
4) Or use another method (such as Graham, McCaughan, and McKee. 1988. Measurement of surface stones. Hydrobiologia 157:85-87) to measure the surface area.

Calculations

1) Follow the equations in the AStandard Methods for Examination of Water and Waste Water≅ (APHA et al. (1992)), the chlorophyll *a* concentration is calculated by using the concentration of pigment in the extract and the recorded surface area of the rocks.

C. CRITERIA

The Province of British Columbia (Nordin, 1985) has set chlorophyll *a* criteria for attached growth in streams to protect recreation and/or aesthetics and aquatic life at 50 and 100 milligrams per square meter, respectively. Values above these levels are known to be detrimental to these uses. These criteria are applied province-wide to naturally growing periphytic algae as opposed to algae growing on artificial substrates. These criteria may be used as guidelines for evaluating problematic periphytic growths in Montana streams.

12.1.2.4 Composition and Structure (Sampling, Sample Analysis, Criteria, Assessment Protocols)

Algae are ubiquitous in Montana surface waters, easy to collect, and represented in unpolluted streams by large numbers of species and individual organisms. Different species are differentially sensitive to a variety of pollutants, including temperature, sediment, nutrients, salts, and toxics. As primary producers, algae are more sensitive to certain pollutants, like nutrients and herbicides, than other aquatic organisms. Measures of the structure of algal associations, such as species diversity and dominance, are sensitive and useful indicators of water pollution and ecological disturbance.

Three levels of assessment are used by the DEQ to evaluate the composition and structure of algal associations:

Level I: Aquatic Plant Field Sheet (APFS)(Section 21.1.1.8)

Level II: Identification of soft-bodied algae to genus; estimated relative abundance of cells in each genus; estimated rank of each genus according to biomass

Level III: Identification of diatoms to species; proportional count yielding percent relative abundance of each species; calculation of diatom association metrics

Each level builds on information generated in the preceding level below. Level I is a prerequisite for Levels II and III and Level II is a prerequisite for Level III assessment.

A. SAMPLING

Microalgae are collected from natural substrates in proportion to the rank of those substrates at the study site as recorded on the Aquatic Plant Field Sheet (Section 21.1.1.8). Collection of microalgae typically involves scraping the entire surface of several rocks, lifting the algal film off from nearshore sediments, and scraping a submerged branch or two. A stainless steel teaspoon is a good all-around tool for collecting microalgae.

Macroalgae are picked by hand in proportion to their abundance at the site. In selecting macroalgae for sampling, the sampler tries to visually distinguish between the various growth forms that represent different algal taxa. Macroalgae are collected both for determining community composition and as substrates for microalgae. The goal is to collect a single composite sample that is a miniature replica of the stand of algae that are present at the study site.

All collections of microalgae and Macroalgae are pooled into a common sample container. Wide-mouth, four ounce (125 ml), plastic jars work well. Enough ambient water should be added to the container to cover the sample. Then enough iodine potassium iodide (Lugol's Solution) should be added to impart a light brown tint to the sample. The purpose of the Lugol's Solution is to retard bacterial decay and selectively stain certain algae for easier identification. (**CAUTION!** If spilled, Lugol's solution will stain clothing and turn paper labels black.)

An identifying label should be affixed to the outside of the container. The label should include stream name and location, the name of the collector, and the date.

Samples may be transported without refrigeration, but they should be kept dark and cold in a refrigerator until they are processed. If samples are stored for a long time, especially if they are stored at room temperature and in daylight, or if they contain a large amount of algae, the Lugol's Solution should be replenished every few weeks.

B. ANALYSIS

Level II -- Soft-Bodied Algae

The sample is poured into a shallow pan and small portions of different macroalgae are removed to a microscope slide. Remainder of the sample is returned to the sample jar and agitated to dislodge epiphytic algae and randomize algal cells and colonies.

Then, using a soda straw or large-bore pipette, a several-drop subsample of microalgae is added to the fragments of macroalgae on the glass slide. A coverslip is placed over the algae subsample, completing a composite wet mount.

The wet mount is scanned under a compound microscope at 200X. Soft-bodied algae are identified to genus, stepping up the magnification to 400X if necessary. After all of the common soft-bodied algae are identified, each genus is ranked according to its estimated contribution to the total algal **biomass** at the site, taking into account the remaining macroalgae and microalgae in the original sample, and information recorded on the Aquatic Plant Field Sheet (APFS) (Section 21.1.1.8). The genus with the most biomass is ranked number 1; the genus with the next most biomass is ranked number 2, and so on. Diatoms are included, but they are ranked as a group (Class Bacillariophyceae) and not as individual genera. Genera that are rated Arare≅ are not ranked.

Genera of soft-bodied algae and diatoms as a group are also rated as to the relative **abundance** of their cells:

R	(rare)	Fewer than 1 cell per field of view at 200X, on the average;
C	(common)	At least one, but fewer than five cells per field of view;
VC	(very common)	Between 5 and 25 cells per field of view;
A	(abundant)	More than 25 cells per field of view, but countable;
VA	(very abundant)	Number of cells per field too numerous to count.

These designations have no counterpart in terms of cells per unit area of stream bottom. Although the density of algae material in each wet mount shall vary, a certain degree of standardization is achieved by the need to provide sufficient separation of cells and passage of light through the mount to allow for the identification of genera and estimation of cell numbers.

The above information should be recorded in a lab notebook, along with information from the sample label, the name of the analyst, the date of the analysis, project name, and other information as needed.

The dominant phylum, indicator taxa, and number of soft-bodied genera may be used to evaluate the biological integrity of the study site in comparison to a control site or ecoregional reference conditions.

Level III -- Diatoms

Digest the remainder of the sample with concentrated acid to remove organic matter and cell contents and prepare a permanent diatom mount according to Procedure 10200D.3 in Standard Methods (APHA et al., 1992).

Next, perform a diatom species proportional count on the permanent mount of between 350 and 450 cells (APHA et al., 1992). The number of cells recorded for each species is divided by the total count and multiplied by 100 to obtain percent relative abundance (PRA). Those species encountered in a floristic scan that precedes the proportional count but not during the count itself are designated with a "p" for "present".

Diatom species and raw counts should be recorded on a bench sheet along with ancillary information. Electronic data storage and programs for calculating PRAs and metrics are also recommended.

In addition to the total number of species counted, five metrics may be calculated from the diatom proportional count PRA data: (1) Shannon diversity index; (2) pollution index; (3) sedimentation index; (4) disturbance index; and (5) similarity index.

Diversity Index. The Shannon diversity index incorporates elements of both dominance (equitability) and species richness. Shannon diversity is less sensitive than species richness to the number of frustules counted.

Pollution Index. The pollution index is based on the decimal fraction of diatoms in each of three pollution tolerance groups: (1) most tolerant; (2) less tolerant; and (3) sensitive. Common Montana diatoms are assigned to one of these three pollution tolerance groups in Section 21.3.1. This fraction is multiplied by the respective group number and the sum of these products is the pollution index. This index shall range from 1.00 (all most-tolerant diatoms) to 3.00 (all sensitive diatoms).

Achnanthes minutissima, a common diatom in Montana streams, has a broad ecological amplitude. It can dominate diatom associations in both very polluted and very pristine streams. It is recommended that *Achnanthes minutissima* be excluded from calculations of the pollution index when it accounts for 3 percent or more of the cells in the proportional count.

Sedimentation Index. This index is equal to the sum of the PRAs for all motile diatom species present in the sample. Most if not all species in the following genera are motile: *Navicula, Nitzschia, Surirella,* and *Cylindrotheca.* The sedimentation index shall yield values ranging from 0.0 to 100.

Disturbance Index. *Achnanthes minutissima* is a common pioneer species in mountain streams and often dominates substrates that are disturbed by either physical abrasion or by chemical pollution. The percent relative abundance (percent dominance) useful index of disturbance, either chemical or physical. PRAs <25 indicate a normal level of disturbance; PRAs between 25 and 50 indicate minor disturbance; PRAs between 50 and 75 indicate moderate disturbance; and PRAs >75 indicate a high level of disturbance.

Similarity Index. This index is the sum of the smaller of the two PRA values for each species that is common to both the control site and the study site or to two study sites. Species restricted to one or the other site are not tallied because the smaller of the two PRA values shall always be zero. Values for this index shall range from 0.0 (totally different communities) to 100 (identical communities).

C. CRITERIA AND ASSESSMENT PROTOCOLS

Two sets of criteria and two assessment protocols are offered for the diatoms: one for screening study sites based on reference conditions established for mountain (high-gradient) and plains (low-gradient) streams (Protocol I), and another for assessing impairment based on conditions at an upstream or tributary control site (Protocol II). Both protocols distinguish among four levels of aquatic life impairment and biological integrity. Protocol I should be used only with metrics calculated from samples collected during the summer index period. Protocol II can be applied to data collected anytime during the year.

If both protocols are used, results of Protocol II should be given more weight. This is because Protocol II is more sensitive to local conditions. However, this is true only if the local control site is relatively unimpaired (rates "good" or "excellent" under Protocol I) and represents the biological potential for the study stream.

Protocol I: Screening Protocol

This protocol assesses biological integrity and aquatic life impairment by comparing metric values from a study site to metric values derived from least-impaired reference streams in the same physiographic province. Separate sets of criteria have been developed for mountain (high-gradient) streams (Table 1) and plains (low-gradient) streams (Table 2). The different criteria for the two types

of streams reflect the natural factors that influence index values: summer temperatures, concentrations of nutrients, sediments, and salts, stream gradient and sedimentation.

Up to four diatom indexes may be used in this protocol: (1) species diversity index; (2) pollution index; (3) sedimentation index; and (4) disturbance index (mountain streams only). Each index is assigned a score based on the value for that index in relation to the criteria in Table 1 or Table 2. The **lowest** score establishes the overall biological integrity and impairment rating for the community of organisms at that site.

Natural stress may result in unusually low diversity index values and high disturbance index values for streams that are pristine in all other respects. This is often true for small mountain streams that have consistently cold water, steep gradients, and low levels of nutrients and light. *Achnanthes minutissima* often dominates the diatom floras of these streams. Mountain streams dominated by this taxon and unimpaired by human activities may have Shannon diversity index values roughly 2.00; mountain streams with diversity index values much lower than 2.00 (i.e., <1.75) are probably impaired.

Protocol II: Control Site Protocol

This protocol compares metric values from a study site to metric values from a local upstream or sidestream control site (Table 3). The control site must be of the same stream order as the study site. In addition to three of the indexes used in Protocol I, this protocol uses the percent similarity index. Protocol II is more sensitive than Protocol I because it compares study sites with local reference sites rather than to generalized regional conditions. The local control site used in Protocol II should rate "good" or "excellent" under Protocol I. Protocol II can be applied year round.
Protocol II recognizes a possible two-way response by diatom diversity to different causes and degrees of impairment. Some mountain streams with naturally low diversity values are known to respond to an increase in sediment and/or nutrients with an **increase** in diversity. These streams also experience an increase in the number of species counted. No intrinsic value is placed on this additional diversity because (1) the species added are more tolerant of pollution than preexisting species, and (2) it represents a deviation from the undisturbed condition for that site.

Metric	Reference	Range of Values	Expected Response
Shannon Species Diversity	Bahls 1979	0.00-5.0+	Decrease[1]
Pollution Index[2]	Bahls 1993	1.00-3.00	Decrease
Siltation Index[3]	Bahls 1993	0.00-90.0+	Increase

1 Shannon diversity and species richness may increase somewhat in naturally nutrient-poor mountain streams in response to slight to moderate increases in nutrients or sediment.

2 This is a composite numeric expression of the pollution tolerances assigned by Lang-Bertalot (1979) to the common diatom species; responds to **organic** pollution only.

3 Computed as the sum of the percent abundances of all species in the genera *Navicula, Nitxschia,* and *Surirella.* These are common genera of a predominantly motile taxa that are able to maintain their positions on the substrate surface in depositional environments.

Disturbance Index4	Barbour et al. 1997	0.00-100.0	Increase
No. Species Counted	Bahls 1979, 1993	0-100+	Decrease[1]
Percent Dominant Species	Barbour et al. 1997	5.0-100.0	Increase
Percent Abnormal Cells	McFarland et al. 1997	0.0-20.0+	Increase
Similarity Index	Whittaker 1952	0.0-80.0+	Decrease

4 Computed as the percent abundance of *Achnanthes minutissima.* This attached taxon typically dominated early successional stages of benthic diatom associations and resists chemical, physical and biological disturbances in the form of metals toxicity, substrate scour by high flows and fast currents.

D. REFERENCES

American Public Health Association, American Water Works Association, and the Water Environment Federation (1992). Standard Methods for the Examination of Water and Wastewater, 18th Edition. A.P.H.A., Washington, D.C.

Britton, L.J., and P.E. Greeson, 1989, Methods for Collection and Analysis of Aquatic Biological and Microbiological Samples. Book 5, Chapter A4, Techniques of Water-Resources Investigations of the United States Geological Survey, U.S.D.I., U.S. Government Printing Office, Washington, D.C.

Cattaneo, A., and G. Roberge. 1991. Efficiency of a Brush Sampler to Measure Periphyton in Streams and Lakes. Canadian Journal of Fish and Aquatic Sciences. 48: 1877-1881.

Nordin, R.N. (1985), Water Quality Criteria for Nutrients and Algae. Water Management Branch, Ministry of Environment, Province of British Columbia, Victoria. Plafkin, J.L., M.T. Barbour, K.D. Porter, S.K. Gross, and R.M. Hughes (1989). Rapid Bioassessment Protocols for Use in Streams and Rivers, EPA/440/4-89/001.

Porter, S.D., T.F. Cuffney, M.E. Gurtz, and M.R. Meador (1993). Methods for Collecting algal Samples as Part of the National Water-Quality Assessment Program, U.S. Geological Survey Open-File Report 93-409.

Stevenson, Dr. R. Jan. University of Louisville. Revision to Rapid Bioassessment Protocols For Use in Streams and Rivers: Periphyton, Benthic, Macroinvertebrates, and Fish. EPA 841-D-97-002. July, 28, 1997.